



Usefulness of Kaposi's Sarcoma-Associated Herpesvirus (KSHV) DNA Viral Load in Whole Blood for Diagnosis and Monitoring of KSHV-Associated Diseases

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Title: Usefulness of Kaposi sarcoma-associated herpesvirus (KSHV)-DNA viral load in whole blood for the diagnosis and monitoring of KSHV-associated diseases.

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Kaposi Sarcoma-Associated Herpesvirus (KSHV) is the etiologic agent of Kaposi's sarcoma (KS) but also in multicentric variant of Castleman disease (MCD) and primary effusion lymphoma (PEL), diseases occurring primarily in HIV-infected patients (1). Several studies have demonstrated that KSHV-DNA levels and virus state in the target cells differ according to the pathology and its severity(2). In peripheral blood from patients with active disease, KSHV-DNA levels were higher from patients with MCD, followed by patients with PEL and then from patients with KS (3). Moreover, variations of KSHV-DNA rates in blood were associated with progression or regression under successful treatment of KS (4, 5) and MCD (6). However recently, Haq and colleagues demonstrated that KSHV plasma levels has a very limited value, the only potential role was the suggestion that an undetectable plasma KSHV exclude a diagnosis of MCD (7).

This retrospective transversal study included 149 patients with KS (111), MCD (32) and PEL (6). One whole blood sample per patient and 4 total effusion fluids from PEL patients were analyzed and obtained at the time of diagnosis. Extracted DNA were amplified by real-time PCR which focus on both ORF73 and albumin genes(8). Quantification was expressed in copies/million cells. GraphPad software was used to perform non-parametric tests: the Mann-Whitney *U*, Spearman rank and Kruskal-Wallis tests.

Patients' characteristics and results are shown in table 1. KSHV-DNA viral load was undetectable in 22% of studied cases or detectable with low levels in KS patients while it was always detectable in MCD and PEL patients. The three KSHV-associated diseases were associated with significantly different levels of KSHV-DNA in whole blood ($p<0.0001$)(Fig. 1). KSHV-DNA rates from MCD's patients were the highest (median, 3.94 log₁₀ copies/10⁶ cells [range, 1.00-7.00]), followed by PEL patients (3.46 log₁₀ copies/10⁶ cells [2.23-4.83]) and

70 finally KS patients ($1.92 \log_{10}$ copies/ 10^6 cells [1.00-5.60]). In patients with KS and MCD,
71 KSHV-DNA levels and CD4 count cells were negatively correlated (respectively $r_s=-0.25$;
72 $p=0.02$ and $r_s=-0.43$; $p=0.02$) confirming the role of immunosuppression in KSHV-diseases
73 development(5). Among PEL patients, KSHV-DNA levels in total effusion fluids were
74 approximately 100 to 1000 times higher than in blood which is in agreement with the
75 physiopathology of the disease but also linked to the high number of KSHV copies per
76 lymphoma cell (2). KSHV-DNA levels in whole blood of patients with MCD were significantly
77 higher than those of KS patients ($p<0.0001$) in contrast to *Haq and al* results in plasma. We
78 know that KSHV virus remain mostly latent in KS and PEL whereas in MCD, about 15% of
79 them are in lytic cycle(2). Moreover, latent and replicating viral KSHV-DNA are presents in
80 cells (9). Thus, plasma samples might underestimate KSHV-DNA levels in peripheral blood
81 compartment in comparison to whole blood, especially in MCD's patients.

82 Our study reinforce that KSHV-DNA biomarker would be helpful to guide diagnosis of and
83 manage KSHV-associated diseases. Even if for KS diagnosis, KSHV-DNA quantification should
84 be interpreted with cautious (about a quarter undetectable), increase levels in blood should
85 trigger KS disease progression or other underlying KSHV-malignancies.

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149 **Table 1: Characteristics’ patients and results of KSHV-DNA status and viral load in**
150 **peripheral blood from the 149 patients included with KSHV-associated diseases**

Disease (No)	Male sex (%)	Median age [range], years	Patients KSHV-DNA status,						Median KSHV-DNA levels, log ₁₀ copies/10 ⁶ cells		
			HIV-infected patients		Detectable, ≥1log ₁₀ copies/10 ⁶ cells		Undetectable, <1log ₁₀ copies/10 ⁶ cells				
			No	Median CD4 count	VIH+	VIH–	VIH+	VIH–	All patients	VIH+	VIH–
			No (%)	[range], cells/mm3, (No)	No (%)	No (%)	No (%)	No (%)	[range] (No)	[range] (No)	[range] (No)
KS (111)	102 (92)	54 [23-93]	76 (68)	210 [1-823] (74)	60/76 (79)	20/28 (71)	16/76 (21)	8/28 (29)	1,92 [1.00-5.60] (111)	2,00 [1.00-5.60] (76)	1,64 [1.00-3.30] (28)
MCD (32)	27 (84)	49 [27-87]	27 (84)	239 [10-920] (27)	26/27 (96)	2/2 (100)	1/27 (4)	0/2 (0)	3,94 [1.00-7.00] (32)	4,12 [1.00-5.82] (27)	5,7 (2)
PEL (6)	6 (100)	58,5 [34-85]	5 (83)	162 [18-450] (5)	4/4 (100)	1/1 (100)	0/4 (0)	0/1 (0)	3,46 [2.23-4.83] (5)	3,62 [4.41-4.83] (4)	2,23 (1)

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152 **NOTE:** KSHV: Kaposi Sarcoma-associated Herpesvirus; KS: Kaposi Sarcoma; MCD: Multicentric Castleman

153 Disease; PEL: Primary Effusion Lymphoma; No: number

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161 **Figure 1 Legend:**

162 **Levels of Kaposi sarcoma-associated herpesvirus (KSHV) in whole blood and effusion fluid**
163 **samples from patients with Kaposi Sarcoma (KS), Castleman multicentric disease (MCD)**
164 **and Primary effusion lymphoma (PEL) at the time of KSHV-associated diseases diagnosis.**

165 **Horizontal lines:** median value (m); **cross:** average; **boxes:** Quartile 1 and Quartile3;

166 **whiskers:** 95% confidence interval; **black circles:** outliers; **Kruskal-Wallis test** ($p<0.0001$);

167 **Mann-Whitney *U* test** ($p<0.016$)

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